PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification [4]:
C07K 15/00, C12P 21/00
G01N 33/577, 33/569, A61K 39/40
// C12N 15/00 (C12P 21/00
C12R 1:91)

A1

(11) International Publication Number: **WO 86/ 02360**

(43) International Publication Date: 24 April 1986 (24.04.86)

(21) International Application Number: PCT/GB85/00471

(22) International Filing Date: 16 October 1985 (16.10.85)

(31) Priority Application Number: 8426469

(32) Priority Date: 19 October 1984 (19.10.84)

(33) Priority Country: GB

(71) Applicant *(for all designated States except US)*: TECHNOLOGY LICENCE COMPANY LIMITED [GB/GB]; 24 Finch Road, Douglas, Isle of Man (GB).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)* : WRIGHT, Bruce, William [GB/GB]; 48 Bush Close, Comberton, Cambridgeshire CB3 7EG (GB). COX, Peter, John [GB/GB]; Church Cottage, Church Road, Chevington, Bury St. Edmunds, Suffolk IP19 5QH (GB). NOYES, Alice, Margaret [GB/GB]; 68 High Street, Willingham, Cambridgeshire CB4 5ES (GB). WIDDOWS, Danny [GB/GB]; 14 Sherbourne Close, Cambridge CB4 1RT (GB).

(74) Agent: GILL JENNINGS & EVERY; 53/64 Chancery Lane, London, WC2A 1HN (GB).

(81) Designated States: AT (European patent), BE (European patent), CH (European patent), DE (European patent), FR (European patent), GB (European patent), IT (European patent), JP, LU (European patent), NL (European patent), SE (European patent), US.

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: MONOCLONAL ANTIBODIES AND THEIR USE

(57) Abstract

Monoclonal antibodies to the genus *Hemophilus*, the labelled antibodies, compositions and kits containing them, and their use in diagnosis of antigen and treatment.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	**GA**	Gabon	**MR**	Mauritania
AU	Australia	**GB**	United Kingdom	**MW**	Malawi
BB	Barbados	**HU**	Hungary	**NL**	Netherlands
BE	Belgium	**IT**	Italy	**NO**	Norway
BG	Bulgaria	**JP**	Japan	**RO**	Romania
BR	Brazil	**KP**	Democratic People's Republic of Korea	**SD**	Sudan
CF	Central African Republic			**SE**	Sweden
CG	Congo	**KR**	Republic of Korea	**SN**	Senegal
CH	Switzerland	**LI**	Liechtenstein	**SU**	Soviet Union
CM	Cameroon	**LK**	Sri Lanka	**TD**	Chad
DE	Germany, Federal Republic of	**LU**	Luxembourg	**TG**	Togo
DK	Denmark	**MC**	Monaco	**US**	United States of America
FI	Finland	**MG**	Madagascar		
FR	France	**ML**	Mali		

MONOCLONAL ANTIBODIES AND THEIR USE

BACKGROUND OF THE INVENTION

Of current interest in the fields of analysis and diagnosis is the use of monoclonal antibodies to determine the presence of antigens or species in specimens such as urine, blood, water, milk, and the like.

More particularly, monoclonal antibodies specific for the antigens or species of Hemophilus are desired which when used will rapidly diagnose the presence of such organisms in specimens.

Divisions have been made among the Hemophilus species. Some of the representative members include Hemophilus influenzae and Hemophilus (or Gardnerella vaginalis.

Hemophilus influenzae causes respiratory infections in children, and it is also the most common cause of bacterial meningitis in children. Hemophilus vaginalis has been shown to be the most common cause of malodorous vaginitis in women. Since specific therapy has also been shown to improve this condition, the precise diagnosis of this condition is now a reasonable consideration. Vaginitis and other vaginal symptoms constitute the most common symptoms bringing women to the physician. The ability of monoclonal antibodies specifically to bind

to antigens of Hemophilus can provide many opportunities for diagnosis and treatment. Such specificity is a most important requirement for proper and accurate analysis and/or diagnosis, particularly in diagnosing the presence of diseases which require prompt treatment.

A wide variety of isotopic and nonisotopic immunoassays have been utilized in conjunction with monoclonal antibodies to test for the presence of an antigenic substance. At the present time, agglutination, immuno-fluorescent, chemiluminescent or fluorescent immunoassay, immuno-electron microscopy, radiometric assay systems, radio immunoassays, and enzyme-linked immunoassays are the most common techniques used with the monoclonal antibodies. Other techniques include bioluminescent, fluorescence polarization, and photon-counting immunoassays.

When utilizing the enzyme-linked immunoassay procedure (EIA), it is necessary to bind, or conjugate, the monoclonal antibody with an enzyme capable of functioning in such assay; such as alkaline phosphatase.

The enzyme-linked monoclonal antibody can then be used in the known enzyme-linked immunosorbent assay procedure to determine the presence

of an antigenic substance.

After the specific antigen is identified, the serotype of the infecting organism can be determined, and appropriate treatment can then be initiated to rapidly and efficiently eliminate the disease.

The production of monoclonal antibodies is now a well-known procedure first described by Kohler and Milstein (Eur. J. Immunol. 6, 292 (1975)). While the general technique of preparing hybridomas and the resultant monoclonal antibodies is understood, it has been found that preparing a specific monoclonal antibody to a specific antigen is difficult, mainly due to the degree of specificity and variations required in producing a particular hybridoma.

SUMMARY OF THE INVENTION

The present invention provides novel monoclonal antibodies for use in accurately and rapidly diagnosing samples for the presence of Hemophilus antigens and/or organisms.

Briefly stated, the present invention comprises monoclonal antibodies specific for an antigen or species of Hemophilus; in particular, the antigens or species of Hemophilus vaginalis (designated as H. vaginalis I, II, III, or IV),

and the antigens or species of Hemophilus influenzae, as well as a monoclonal antibody broadly cross-reactive with an antigen for each species of the genus Hemophilus.

The invention also comprises labeled monoclonal antibodies for use in diagnosing the presence of the Hemophilus antigens, each comprising a monoclonal antibody against one of the above-mentioned antigens to Hemophilus or to a particular species thereof and linked thereto an appropriate label. The label can be chosen from the group consisting of a radioactive isotope, enzyme, fluorescent compound, chemiluminescent compound, bioluminescent compound, ferromagnetic atom, or particle, or any other label.

The invention further comprises the process for diagnosing the presence of Hemophilus antigens or organisms in a specimen comprising contacting said specimen with the labeled monoclonal antibody in an appropriate immunoassay procedure.

Additionally, the invention is also directed to a therapeutic composition comprising a monoclonal antibody for an antigen of Hemophilus and a carrier or diluent, as well as kits containing at least one labeled monoclonal antibody to an antigen of a Hemophilus.

DETAILED DESCRIPTION

The monoclonal antibodies of the present invention are prepared by fusing spleen cells, from a mammal which has been immunized against the particular Hemophilus antigen, with an appropriate myeloma cell line, preferably NS0 (uncloned), P3NS1-Ag4/1, or Sp2/0 Ag14. The resultant product is then cultured in a standard HAT (hypoxanthine, aminopterin, and thymidine) medium. Screening tests for the specific monoclonal antibodies are employed utilizing immunoassay techniques which will be described below.

The immunized spleen cells may be derived from any mammal, such as primates, humans, rodents (i.e., mice, rats, and rabbits), bovine, ovine, canine, or the like, but the present invention will be described in connection with mice. The mouse is first immunized by injection of the particular Hemophilus antigen chosen generally for a period of approximately eleven weeks. When the mouse shows sufficient antibody production against the antigen, as determined by conventional assay, it is given a booster injection of the appropriate Hemophilus antigen, and then killed so that the immunized spleen may be removed. The fusion can then be carried out utilizing

immunized spleen cells and an appropriate myeloma cell line.

The fused cells yielding an antibody which give a positive response to the presence of the particular Hemophilus antigen are removed and cloned utilizing any of the standard methods. The monoclonal antibodies from the clones are then tested against standard antigens to determine their specificity for the particular Hemophilus antigen. The monoclonal antibody selected, which is specific for the particular Hemophilus antigen or species, is then bound to an appropriate label.

Amounts of antibody sufficient for labeling and subsequent commercial production are produced by the known techniques, such as by batch or continuous tissue culture or culture in vivo in mammals, such as mice.

The monoclonal antibodies may be labeled with a multitude of different labels, such as enzymes, fluorescent compounds, luminescent compounds, radioactive compounds, ferromagnetic labels, and the like. The present invention will be described with reference to the use of an enzyme labeled monoclonal antibody. Some of the enzymes utilized as labels are alkaline

phosphatase, glucose oxidase, galactosidase, peroxidase, or urease, and the like.

Such linkage with enzymes can be accomplished by any one of the conventional and known methods, such as the Staphylococcal Protein A method, the glutaraldehyde method, the benzoquinone method, or the periodate method.

Once the labeled monoclonal antibody is formed, testing is carried out employing one of a wide variety of conventional immunoassay methods. The particular method chosen will vary according to the monoclonal antibody and the label chosen. At the present time, enzyme immunoassays are preferred due to their low cost, reagent stability, safety, sensitivity, and ease of procedure. One example is enzyme-linked immunosorbent assay (EIA). EIA is a solid phase assay system which is similar in design to the radiometric assay, but which utilizes an enzyme in place of a radioactive isotope as the immunoglobulin marker.

Fluorescent-immunoassay is based on the labeling of antigen or antibody with fluorescent probes. A nonlabeled antigen and a specific antibody are combined with identical fluorescently labeled antigen. Both labeled and unlabeled

antigen compete for antibody binding sites. The amount of labeled antigen bound to the antibody is dependent upon, and therefore a measurement of, the concentration of nonlabeled antigen. Examples of this particular type of fluorescent-immunoassay would include heterogenous systems such as Enzyme-Linked Fluorescent Immunoassay, or homogeneous systems such as the Substrate Labeled Fluorescent Immunoassay. The most suitable fluorescent probe, and the one most widely used is fluorescein. While fluorescein can be subject to considerable interference from scattering, sensitivity can be increased by the use of a fluorometer optimized for the probe utilized in the particular assay and in which the effect of scattering can be minimized.

In fluorescence polarization, a labeled sample is excited with polarized light and the degree of polarization of the emitted light is measured. As the antigen binds to the antibody its rotation slows down and the degree of polarization increases. Fluorescence polarization is simple, quick, and precise. However, at the present time its sensitivity is limited to the micromole per liter range and upper nanomole per liter range with respect to antigens

in biological samples.

Luminescence is the emission of light by an atom or molecule as an electron is transferred to the ground state from a higher energy state. In both chemiluminescent and bioluminescent reactions, the free energy of a chemical reaction provides the energy required to produce an intermediate reaction or product in an electronically excited state. Subsequent decay back to the ground state is accompanied by emission of light. Bioluminescence is the name given to a special form of chemiluminescence found in biological systems, in which a catalytic protein or enzyme, such as luciferase, increases the efficiency of the luminescent reaction. The best known chemiluminescent substance is luminol.

A further aspect of the present invention is a therapeutic composition comprising one or more of the monoclonal antibodies to the particular Hemophilus antigen or species, as well as a pharmacologically acceptable carrier or diluent. Such compositions can be used to treat humans and/or animals afflicted with some form of Hemophilus infections and they are used in amounts effective to cure; an amount which will vary widely dependent upon the individ-

ual being treated and the severity of the infection.

One or more of the monoclonal antibodies can be assembled into a diagnostic kit for use in diagnosing for the presence of an antigen, antigens, or species of Hemophilus in various specimens. It is also possible to use the broadly cross-reactive monoclonal antibody which can identify the genus Hemophilus alone or as part of a kit containing antibodies that can identify other bacterial genera or species of Hemophilus and/or other bacteria.

In the past there have been difficulties in developing rapid kits because of undesirable cross-reactions of specimens with antiserum. The use of monoclonal antibodies can eliminate these problems and provide highly specific and rapid tests for diagnosis. A rapid and precise kit could replace or augment existing tests and permit early direct therapy using precise antibiotics. Avoiding multiple antibiotics or more expensive or hazardous antibiotics would represent substantial patient and hospital savings. Additionally, a kit can be used on an out-patient basis. At present the lack of a rapid test giving "same day" answers may delay

the initiation of treatment until the patient has developed more severe symptoms or may require the initiation of more costly therapy in a sick patient. A test that would return results within an hour or two would be a substantial convenience to patients.

In addition to being sold individually, the kit could be included as a component in a comprehensive line of compatible immunoassay reagents sold to reference laboratories to detect the species and serotypes of Hemophilus.

One preferred embodiment of the present invention is a diagnostic kit comprising at least one labeled monoclonal antibody against a particular Hemophilus antigen or species, as well as any appropriate stains, counterstains, or reagents. Further embodiments include kits containing at least one control sample of a Hemophilus antigen and/or a cross-reactive labeled monoclonal antibody which would detect the presence of any of the Hemophilus organisms in a particular sample. Specific antigens to be detected in this kit include the antigens of Hemophilus vaginalis (applicant has further divided this species into four subgroups: Hemophilus vaginalis I, II, III, or IV), and Hemophi-

lus influenzae.

Monoclonal diagnostics which detect the presence of Hemophilus antigens can also be used in periodic testing of water sources, food supplies and food processing operations. Thus, while the present invention describes the use of the labelled monoclonal antibodies to determine the presence of a standard antigen, the invention can have many applications in diagnosing the presence of antigens by determining whether specimens such as urine, blood, stool, water and milk contain the particular Hemophilus antigen. More particularly, the invention could be utilised as a public health and safety diagnostic aid, whereby specimens such as water or food could be tested for possible contamination.

References to Hemophilus herein should also be read as references to Gardnerella, in general, and specifically in connection with Hemophilus vaginalis.

The invention will be further illustrated in connection with the following Examples which are set forth for the purposes of illustration only and not by way of limitation.

In the Examples:

API = Analytical Profile Index (ref. Ayerst Labs)

DMEM = Dulbecco's Modified Eagles Medium

FCS = Foetal Calf Serum

PBS = phosphate-buffered saline

% T refers to vaccine concentration measured in a 1 cm light path

Monoclonal antibodies of the present invention are prepared generally according to the method of Koehler and Milstein, Eur. J. Immunol. 6 (1975) 292.

EXAMPLE 1

A. Antigen Preparation

Gardnerella vaginalis is obtained from the National Collection of Type Culture (NCTC accession No. 10915) and

tested by standard biochemical methods of microbial identification to confirm its identity (using API profiles). The antigen is removed from the lyophile, grown on blood agar, and tested by API to confirm its identity and purity. It is then transferred for growth into Todd-Hewitt broth in 10% CO_2. After incubation, the cells are harvested by centrifugation, and washed three times in saline. They are then resuspended in 1% formol saline.

B. Animal Immunisation

Six balb/c mice are injected with the prepared antigen. They are given one intraperitoneal injection per week for 3 weeks and then, after 1, 1 and 2 week intervals, an intravenous injection (0.05 ml 80% T vaccine) of vaccine prepared as above. The mice are bled approximately six days after the last injection and the serum tested for antibodies by assay. A conventional assay used for this serum titer testing is the enzyme-linked immunosorbent assay system. When the mice show antibody production after this regimen, generally a positive titer of at least 10,000, a mouse is selected as a fusion donor and given a booster injection (0.05 ml 80% T vaccine) intravenously, three days prior to splenectomy.

C. Cell Fusion

Spleen cells from the immune mice are harvested three days after boosting, by conventional techniques. First, the donor mouse selected is killed and surface-sterilised by immersion in 70% ethyl alcohol. The spleen is then removed and immersed in approximately 2.5 ml DMEM to which has been added 3% FCS. The spleen is then gently homogenised in a LUX homogenising tube until all cells have been released from the membrane, and the cells are washed in 5 ml 3% FCS-DMEM. The cellular debris is then allowed to settle and the spleen cell

suspension placed in a 10 ml centrifuge tube. The debris is then rewashed in 5 ml 3% FCS-DMEM. 50 ml suspension are then made in 3% FCS-DMEM.

The myeloma cell line used is NSO (uncloned), obtained from the MRC Laboratory of Molecular Biology in Cambridge, England. The myeloma cells are in the log growth phase, and rapidly dividing. Each cell line is washed using, as tissue culture medium, DMEM containing 3% FCS.

The spleen cells are then spun down at the same time that a relevant volume of myeloma cells are spun down (room temperature for 7 minutes at 600 g), and each resultant pellet is then separately resuspended in 10 ml 3% FCS-DMEM. In order to count the myeloma cells, 0.1 ml of the suspension is diluted to 1 ml and a haemacytometer with phase microscope is used. In order to count the spleen cells, 0.1 ml of the suspension is diluted to 1 ml with Methyl Violet-citric acid solution, and a haemacytometer and light microscope are used to count the stained nuclei of the cells.

1×10^8 Spleen cells are then mixed with 5×10^7 myeloma cells, the mixture washed in serum-free DMEM high in glucose, and centrifuged, and all the liquid removed. The resultant cell pellet is placed in a 37°C water-bath. 1 ml of a 50 w/v solution of polyethylene glycol 1500 (PEG) in saline Hepes, pH approximately 7.5, is added, and the mixture gently stirred for approximately 1.5 minutes. 10 ml serum-free tissue culture medium DMEM are then slowly added, followed by up to 50 ml of such culture medium, centrifugation and removal of all the supernatant, and resuspension of the cell pellet in 10 ml of DMEM containing 18% by weight FCS.

10 µl of the mixture are placed in each of 480 wells of standard multiwell tissue culture plates. Each well contains 1.0 ml of the standard HAT medium (hypoxanthine,

aminopterin and thymidine) and a feeder layer of Balb/c macrophages at a concentration of 5×10^4 macrophages/well.

The wells are kept undisturbed, and cultured at 37°C in 9% CO_2 air at approximately 100% humidity. The wells are analysed for growth, utilising the conventional inverted microscope procedure, after about 5 to 10 days. In those wells in which growth is present in the inhibiting HAT medium, screening tests for the specific monoclonal antibody are made utilising the conventional enzyme immunoassay screening method described below. Somewhere around 10 days to 14 days after fusion, sufficient antibody against the antigen may develop in at least one well.

D. Cloning

From those wells which yielded antibody against the antigen, cells are removed and cloned using the dilution method. In limiting dilution, dilutions of cell suspensions in 18% FCS-DMEM + Balb/c mouse macrophages were made to achieve 1 cell/well and half cell/well in a 96-well microtitre plate. The plates were incubated for 7-14 days at 37 C, 95% RH, 7-9% CO_2 until semi-confluent. The supernatants were then assayed for specific antibody by the standard enzyme immunosorbent assay.

The clones may be assayed by the enzyme immunoassay method to determine antibody production.

E. Monoclonal Selection

The monoclonal antibodies from the clones are screened by the standard techniques for binding to the antigen, prepared as in the immunisation, and for specificity in a test battery of the class bearing different antigens. Specifically, a grid of microtiter plates containing a representative selective of organisms is prepared, boiled, and utilised as a template to define

the specificity of the parent group. The EIA immunoassay noted above may be used.

The monoclonal is specific to Gardnerella vaginalis strains NCTC 10915, 17.4 and Corpus, and negative to E. coli, Pseudomonas, Klebsiella, Hemophilus and Shigella.

F. Antibody Production and Purification

Balb/c mice were primed with pristane for at least 7 days, and were then injected with 10^7 cells of the monoclonal antibody-producing cell line. Ascitic fluid was harvested when the mice were swollen with fluid but still alive. The fluid was centrifuged at 1200 g for approximately 10 minutes, the cells discarded and the antibody-rich ascites collected and stored at -20 C.

Ascites fluid was filtered through glass wool and centrifuged at 30,000 g for 10 minutes. The ascites was then diluted with twice its own volume of cold phosphate buffer (0.1M sodium phosphate, pH 8.2). The diluted ascites was applied to a 2 ml column of Protein A-Sepharose, previously equilibrated with phosphate buffer. The column was washed with 40 ml of phosphate buffer. The monoclonal antibody was eluted with citrate buffer (0.1M sodium citrate, pH 3.5) into sufficient 1M TRIS buffer, pH 9.0 to raise the pH immediately to about 7.5. The eluate was dialysed in PBS, pH 7.4, at 4 C and stored at -20 C.

An alternative procedure for antibody purification was also conducted: ascites fluid was filtered through glass wool and centrifuged at 30,000 g for 10 minutes. The ascites was then stirred at +4 C and an equal volume of cold, saturated ammonium sulphate added slowly. The mixture was stirred for a further 30 minutes after the addition was complete. The precipitate was harvested by centrifugation at 10,000 g for 10 minutes. The precipitate was dissolved in a minimum volume of cold phosphate/EDTA buffer (20 mM sodium phosphate, 10 mM

EDTA, pH 7.5, + 0.02% sodium azide). The solution was dialysed vs 2 x 1000 ml of the same buffer at +4 C. The dialysed, redissolved precipitate was centrifuged at 30,000 g for 10 minutes and applied to a 10 ml column of DEAE-cellulose, previously equilibrated in phosphate/EDTA buffer. The monoclonal antibody was eluted with phosphate/EDTA buffer.

G. Enzyme-Monoclonal Linkage

The monoclonal antibody specific against the antigen, prepared as above, is linked to an enzyme, viz. highly-purified alkaline phosphatase.

24 mg alkaline phosphatase (Sigma Type VII-T) were dialysed against 2 x 500 ml of 0.25 M sodium phosphate buffer, pH 6.0, at +4 C. 18 mg p-benzoquinone were dissolved in 0.6 ml warm AR ethanol, and added to the dialysed alkaline phosphatase. The benzoquinone/alkaline phosphatase mixture was left in the dark at room temperature for 1 hour. Unreacted benzoquinone and reaction by-products were then removed and the buffer exchanged by gel filtration on a Pharmacia PD-10 (Sephadex G-25M) column previously equilibrated in 0.15M sodium chloride. The benzoquinone-activated alkaline phosphatase thus produced was sufficient for six 1.5 mg antibody conjugations. Monoclonal antibody was dialysed against 2 x 500 ml of 0.15M sodium chloride at +4 C. Dialysed antibody was added to 4 mg of benzoquinone-activated alkaline phosphatase and immediately followed by sufficient 1M sodium bicarbonate to give a final concentration of 0.1M. The conjugation mixture was left in the dark at +4 C for 48 hours. Sufficientl 1M lysine was then added to give a final concentration of 0.1M. After 2 hours in the dark at room temperature, the conjugate was dialysed against 2 x 1000 ml PBS + 0.02% sodium azide at +4 C. An equal volume of glycerol was added. The conjugate was sterile-filtered through a 0.22

µm membrane filter into a sterile amber vial, and stored at +4 C.

EXAMPLE 2

The general procedure of Example 1 was followed, but with various differences. The antigen was Hemophilus influenzae type b, NCTC 7279. The bacteria were grown in chocolate agar and harvested. The organisms were suspended in formol saline. The animals were immunised intramuscularly (in Complete Freunds Adjuvant) and, 4 weeks later, intravenously.

The monoclonal was specific to the H. influenzae type b antigen, and negative to other strains of Hemophilus, Neisseria, E coli, Salmonella, Pseudomonas, Serratia, Enterobacter and Gardnerella.

In antibody production, cells of the monoclonal antibody-producing cell line were grown in batch tissue culture. DMEM-10% FCS was used to support growth in mid-log phase, to 1 litre volume. The culture was then allowed to overgrow, to allow maximum antibody production. The culture was then centrifuged at 1200 g for approximately 10 minutes, the cells discarded and the antibody-rich supernatant collected.

In antibody purification, to one litre of culture supernatant were added 100 ml of 1.0M TRIS buffer, pH 8.2. The TRIS buffered supernatant was applied at a flow rate of 1 ml/min to a 1 ml column of Protein A-Sepharose, previously equilibrated with 0.1M TRIS buffer, pH 8.2. The column was then washed with 40 ml of 0.1M TRIS buffer. The monoclonal antibody was eluted with citrate buffer (0.1M sodium citrate, pH 3.5) into sufficient 1M TRIS buffer, pH 9.0, to raise the pH immediately to about 7.5. The eluate was dialysed in PBS, pH 7.4, at 4 C, and stored at -20 C.

The sub-class was IgG2a.

EXAMPLE 3

The general procedure of Example 1 may be followed to produce a monoclonal antibody broadly cross-reactive with an antigen of all species of the genus Gardnerella (or Hemophilus).

Tests using the present invention are superior to existing tests, based on the following advantages: (i) greater accuracy; (ii) same day results, within an hour or two; (iii) reduction in amount of skilled labour required to administer laboratory procedures, resulting in reduced labour costs; (iv) reduction in laboratory time and space used in connection with tests, resulting in reduced overhead expenses; and (v) improved therapy based upon early, precise diagnosis.

While the invention has been described in connection with certain preferred embodiments, it is not intended to limit the scope of the invention to the particular form set forth but, on the contrary, it is intended to cover such alternatives, modifications, and equivalents as may be included within the spirit and scope of the invention as defined by the appended claims.

WHAT IS CLAIMED IS:

1. A monoclonal antibody specific for an antigen or species of Hemophilus.

2. The antibody of Claim 1 specific to the antigen or antigens of Hemophilus vaginalis.

3. The antibody of Claim 1 specific to the antigen or antigens of Hemophilus vaginalis I.

4. The antibody of Claim 1 specific to the antigen or antigens of Hemophilus vaginalis II.

5. The antibody of Claim 1 specific to the antigen or antigens of Hemophilus vaginalis III.

6. The antibody of Claim 1 specific to the antigen or antigens of Hemophilus vaginalis IV.

7. The antibody of Claim 1 specific to the antigen or antigens of Hemophilus influenzae, e.g. BI, BII, BIII or BIV.

8. A monoclonal antibody broadly cross-reactive with an antigen of all species of the genus Hemophilus.

9. A labeled monoclonal antibody consisting essentially of a monoclonal antibody of Claims 1-8 and an appropriate label.

10. The labeled monoclonal antibody of Claim 9, wherein said label is a member of the group selected from a radioactive isotope, enzyme, fluorescent compound, bioluminescent compound, chemiluminescent compound, or ferromagnetic atom, or particle.

11. The labeled monoclonal antibody of Claim 10, wherein said label is an enzyme capable of conjugating with a monoclonal antibody and of being used in an enzyme-linked immunoassay procedure.

12. The labeled monoclonal antibody of Claim 11, wherein said enzyme is alkaline phosphatase, glucose oxidase, galactosidase, or peroxidase.

13. The labeled monoclonal antibody of Claim 10, wherein said label is a fluorescent compound or probe capable of being used in an immuno-fluorescent or fluorescent immunoassay procedure, enzyme fluorescent immunoassay, or fluorescence polarization immunoassay, photon counting immunoassay, or the like procedure.

14. The labeled monoclonal antibody of Claim 13, wherein said fluorescent compound or probe is fluorescein.

15. The labeled monoclonal antibody of Claim 10, wherein said label is a chemiluminescent compound capable of being used in a luminescent or enzyme-linked luminescent immunoassay.

16. The labeled monoclonal antibody of Claim 15, wherein such chemiluminescent compound is luminol or a luminol derivative.

17. The labeled monoclonal antibody of Claim 10, wherein said label is a bioluminescent compound capable of being used in an appropriate bioluminescent immunoassay.

18. The labeled monoclonal antibody of Claim 17, wherein such bioluminescent compound is luciferase or a luciferase derivative.

19. A process for diagnosing for the presence of an antigen of Hemophilus in a specimen comprising contacting at least a portion of said specimen with a labeled monoclonal antibody of Claim 9 in an immunoassay procedure appropriate for said label.

20. The process of Claim 19, wherein the appropriately labeled immunoassay procedure is selected from immuno-fluorescent or fluorescent immunoassay, immuno-electron microscopy, radiometric assay systems, enzyme-linked immunoassays, fluorescence polarization, photon-counting bioluminescent, or chemiluminescent immunoassay.

21. The process of Claim 20, wherein said label is an enzyme capable of being used in an enzyme-linked immunoassay procedure.

22. The process of Claim 21, wherein said enzyme is selected from alkaline phosphatase, glucose oxidase, galactosidase, or peroxidase.

23. The process of Claim 20, wherein said label is a fluorescent compound or probe capable of being used in an immuno-fluorescent or fluorescent immunoassay procedure, enzyme fluorescent immunoassay, or fluorescence polarization immunoassay, or photon-counting immunoassay, or the like procedure.

24. The process of Claim 23, wherein said fluorescent compound or probe is fluorescein.

25. The process of Claim 20, wherein said label is a chemiluminescent compound capable of being used in a luminescent or enzyme-linked luminescent immunoassay.

26. The process of Claim 25, wherein said chemiluminescent compound is luminol or a luminol derivative.

27. The process of Claim 20, wherein said label is a bioluminescent compound capable of being used in a bioluminescent or enzyme-linked bioluminescent immunoassay.

28. The process of Claim 27, wherein said bioluminescent compound is luciferase or a luciferase derivative.

29. A therapeutic composition comprising one or more of the monoclonal antibodies in Claims 1-8 and a pharmaceutically acceptable carrier or diluent.

30. A therapeutic composition comprising one or more of the labeled monoclonal antibodies in Claim 9 and a pharmaceutically acceptable carrier or diluent.

31. A method of treating Hemophilus infections comprising administering an effective amount of a monoclonal antibody of Claims 1-8.

32. A kit for diagnosing for the presence of an antigen or species of Hemophilus in a diagnostic specimen comprising at least one monoclonal antibody of Claims 1-8.

33. The kit of Claim 32, wherein said at least one antibody is labeled.

34. The kit of Claim 33, wherein said at least one monoclonal antibody is labeled with a fluorescent compound.

35. The kit as in Claim 33, wherein said at least one monoclonal antibody is labeled with an enzyme.

36. The kit as in Claim 33, wherein said at least one monoclonal antibody is labeled with a member of the group consisting of a radioactive isotope, chemiluminescent compound, bioluminescent compound, ferromagnetic atom, or particle.

37. The kit of Claims 33, 34, 35, and 36 additionally containing at least one known Hemophilus antigen as a control.

38. The kit of Claims 33, 34, 35, 36, and 37 containing each known antigen of Hemophilus vaginalis, and/or Hemophilus influenzae.

39. The kit of Claims 33, 34, 35, 36, and 37 containing the antigens of Hemophilus vaginalis.

40. The kit of Claims 33, 34, 35, 36, and 37 containing the antigens of Hemophilus influenzae.

41. A kit for diagnosing for the presence of an antigen or species of Hemophilus in a diagnostic specimen comprising at least one monoclonal antibody of Claims 1-8 and a control.

42. The kit of Claim 41, wherein said at least one antigen is labeled and said control is at least one known antigen of Hemophilus.

43. A kit for diagnosing for the presence of a Hemophilus infection comprising at least one monoclonal antibody of Claims 1-8.

44. The kit of Claim 43, wherein said at least one monoclonal antibody is labeled.

INTERNATIONAL SEARCH REPORT

International Application No PCT/GB 85/00471

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC[4]: C 07 K 15/00; C 12 P 21/00; G 01 N 33/577; G 01 N 33/569; A 61 K 39/40 // C 12 N 15/00; (C 12 P 21/00; C 12 R 1:91)

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
IPC[4]	C 12 P G 01 N A 61 K

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category [10]	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
X Y	US, A, 4455296 (E.J. HANSEN et al.) 19 June 1984, see claims 13-19; column 10, lines 52-60; column 12, lines 8-27	1,7,8,29,30 32 2-6,9-28, 33-44
Y	Infection and Immunity, volume 18, no. 2, November 1977, Washington, D.C., (US) M.F. Smaron et al.:"Immunological and chemical characterization of the extracellular antigens from corynebacterium vaginale", see page 356, abstract; page 359, left-hand column, lines 10-20; page 362, left-hand column, lines 12-22	2-6,9-28, 33-44
Y	US, A, 4461829 (A.C. GREENQUIST) 24 July 1984, see claims 1-3; column 2, lines 8-40; column 10, lines 17-26,51	9-28,33-44
A	R.E. Buchanan et al., co-editors,"Bergey's manual of determinative bacteriology", 8th edition, published 1975, The Williams & Wilkins Company, Baltimore, (US) pages 368-370, see page 368, lines 33-39	2-6

* Special categories of cited documents: [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
3rd February 1986	19 FEV. 1986
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	M. VAN MOL

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

V. [X] OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE [1]

This International search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. [X] Claim numbers 31, because they relate to subject matter not required to be searched by this Authority, namely:

PCT Rule 39.1(iv): Methods for treatment of the human or animal body by surgery or therapy as well as diagnostic methods.

2. [ ] Claim numbers....., because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claim numbers....., because they are dependent claims and are not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. [ ] OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [2]

This International Searching Authority found multiple inventions in this international application as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.

2. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

4. [ ] As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

[ ] The additional search fees were accompanied by applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON

INTERNATIONAL APPLICATION NO. PCT/GB 85/00471 (SA 10954)

This Annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on 12/02/86

The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US-A- 4455296	19/06/84	None	
US-A- 4461829	24/07/84	CA-A- 1190461	16/07/85